THE EFFECT OF POLARIZATION ON THE ELECTRICAL RESPONSE OF A MUSCLE FIBER

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The dependence of the electrical response of muscle and nerve fibers on their state of polarization can give information concerning the development and spread of excitation in various synaptic structures. Coombs, Eccles and Fatt [3], using a double-barrelled, intracellular electrode, have shown that when motor neurones of the spinal cord are polarized there is a change in the transmission of excitation from one part of the nerve cell to another. Benoit and Coraboeuf [2] found a change in the amplitude of the action potentials of a muscle fiber was caused by polarization.

We have used intracellular polarization, and have led off potentials from the fiber through double-barrelled intracellular microelectrodes to record postsynaptic and spike potentials evoked by indirect stimulation.

METHOD

The experiments were carried out on isolated nerve-muscle preparations of the sartorius muscle of the grass frog. Stimulation was produced by single or repeated square-wave pulses from an electronic stimulator. The muscle potentials were led off from double-barrelled intracellular microelectrodes, as we have described in detail previously [1]. The electrodes were filled with 3 M KCl solution. The diameter of the tip was 0.5 - 1μ, and the resistance was 2-15 Mohm. One of the microelectrodes was connected to the grid of the input cathode-follower of a D. C. amplifier, and the other was connected through a 100 Mohm resistance to a source of direct current. Because of the common resistance to the two channels of the intracellular microelectrode and the capacitative connection between the channels, passing current through one channel caused a considerable fall of potential across the common resistance of the microelectrode. It was therefore difficult to determine a genuine change of membrane potential, and we studied only the magnitude of the polarizing current measured by means of an M-95 galvanometer. Currents used ranged from $1 \cdot 10^{-8}$ to $5 \cdot 10^{-7}$ a. Currents of the order of $2\text{-}5 \cdot 10^{-7}$ a were suprathreshold for some of the fibers.

RESULTS

Hyperpolarization of a muscle fiber caused by a current directed inward into the cell caused an increase in the amplitude of the action potential. The increase was from 120 to 130%, depending on the strength of the hyperpolarizing current. On the other hand, depolarization of the cell by an outwardly directed current caused a considerable fall in the spike potential. After the polarizing current had been switched off, the amplitude of the spike discharge returned to its original value.

Polarization caused changes not only in the spike but also in the electrotonic after potentials. Hyperpolarization caused an increase in the negative after potentials, while depolarization reduced them. These results confirm those of Benoit and Coraboeuf [2] on the frog muscle.

The relationship between the amplitude of the spike potentials and the degree of polarization of the cell is determined to a large extent by the ionic constitution of the surrounding medium. A calcium concentration is the most important. A reduction in the number of calcium ions had the effect that a polarizing current of a given intensity caused greater than normal change in amplitude (Fig. 1).

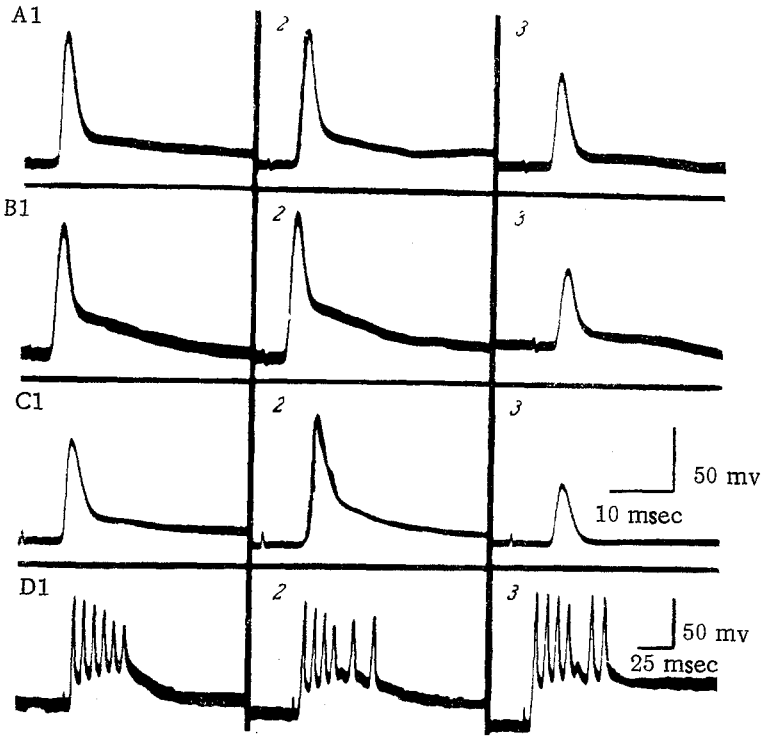

Fig. 1. The effect of polarization on spike potentials. Potentials from the nerveless part of a fiber. A) Normal calcium ion concentration: 1) normal; 2) hyperpolarizing current of $7 \cdot 10^{-8}$a; 3) depolarizing current of $8 \cdot 10^{-8}$ a; B) reduction of the calcium concentration by half: 1) normal; 2) hyperpolarizing current of $7 \cdot 10^{-8}$ a; 3) depolarizing current of $5 \cdot 10^{-8}$ a; C) calcium concentration reduced to 10%; 1) normal; 2) hyperpolarizing current of $7 \cdot 10^{-8}$ a; 3) depolarization with a current of $7 \cdot 10^{-8}$ a; D) various responses to veratrine: 1) normal; 2) hyperpolarization with current of $5 \cdot 10^{-8}$ a; 3) hyperpolarizing current of $1 \cdot 10^{-7}$ a.

The effect influenced both the increase of amplitude due to hyperpolarization and its reduction by depolarization. The marked increase in the sensitivity of the muscle fiber to polarization was noted even when the calcium ion concentration was reduced to one half. The most marked changes occured after reducing it to 10% of the normal value, or after eliminating calcium ions completely from the Ringer solution (see Fig. 1). The results of these experiments therefore agree with those obtained by Frankenhaeuser and Hodgkin [5] using the giant axon of the squid and by Frankenhaeuser [6] on the frog nerve.

Because anelectronic polarization of the cell caused a marked increase in the negative after-potential, it was pertinent to find how negative after-potentials increased by the action of drugs would be affected. We therefore studied the effect of polarization on the negative after-potential, after treatment with veratrine.

Veratrine in a concentration of $1 - 2 \cdot 10^{-6}$ caused a marked increase in the amplitude and duration of the after-potential, and as a consequence, a number of spike potentials developed at its summit. After the muscle had been treated with veratrine, the effect of a hyperpolarizing current was to cause a still greater increase in the amplitude of the after-potential, and the amplitude of the numerous discharges also increased. Similar results were obtained for the numerous responses developing as a consequence of increasing the after-potentials in a muscle fiber placed in a solution deficient in calcium ions.

Thus, increased polarization causes an increase both in transmitted and in electrotonic after-potentials.

To determine the effect of cell polarization on post-synaptic potentials, the microelectrode was placed in the region of the end-plate, as determined by the presence of "miniature end-plate potentials."

According to Fatt and Katz [4], anodic polarization of the subsynaptic region of the end-plate of a muscle fiber causes an increase in the amplitude of the end-plate potential, whereas depolarization produced the opposite effect (Fig. 2).

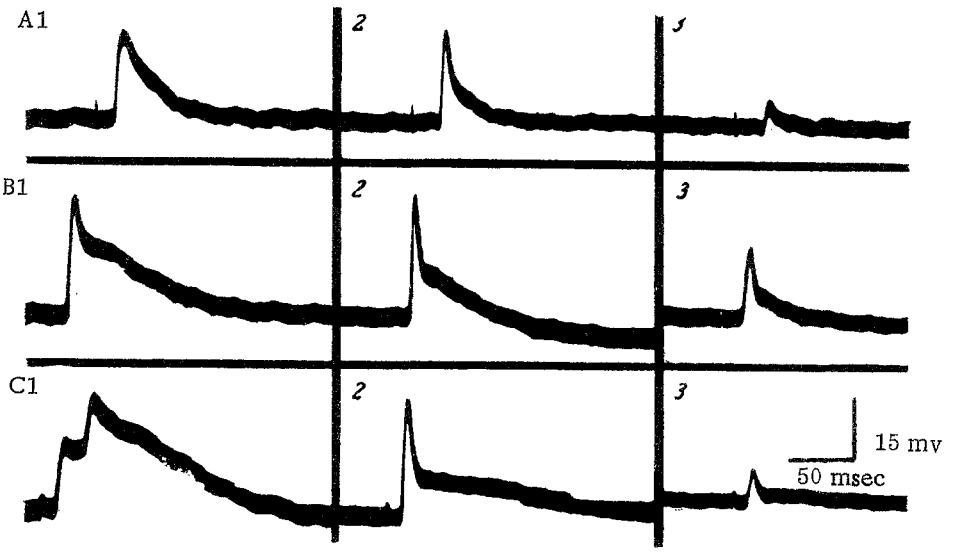

Fig. 2. The effect of polarization on end-plate potentials. A) Normal calcium ion concentration: 1) normal; 2) hyperpolarizing current of $8 \cdot 10^{-8}$ a; 3) depolarizing current of $7 \cdot 10^{-8}$; B) calcium concentration reduced to 10%: 1) normal; 2) hyperpolarizing current of $7 \cdot 10^{-8}$ a; 3) depolarizing current of $7 \cdot 10^{-8}$ a; C) after adding phenyldiguanidine ($4 \cdot 10^{-6}$ a): 1) normal; 2) hyperpolarizing current of $7 \cdot 10^{-8}$ a; 3) depolarizing current of $7 \cdot 10^{-8}$ a.

Unlike the spike potentials of a muscle fiber, during hyperpolarization there was always a shortening of the descending phase of the end-plate potential. This effect of hyperpolarization was particularly well-shown when the end-plate potential was recorded at a reduced calcium ion concentration, or when $4 - 5 \cdot 10^{-6}$ phenyldiguanidine was added to the surrounding medium. The end-plate potentials of such muscle fibers usually had a long negative plateau on the descending portion and its shape was similar to that of the negative after-potential of a spike discharge. After switching on the hyperpolarizing current, besides an increase in amplitude of the ascending portion of the end-plate potential, there was also a marked reduction of the negative plateau of the descending portion. This effect was present even when the hyperpolarization was produced by threshold currents of $1 - 3 \cdot 10^{-8}$ a. Then the reduction of the plateau took place without any change in the amplitude of the ascending portion of the end-plate potential. In some cases, after reduction of the calcium ion concentration or the addition of phenyldiguanidine, the end-plate potential not only was increased in duration, but also took on a more complex form, and additional oscillations occurred in the descending portion. Anodic polarization completely eliminated this additional negative oscillation.

On account of the marked difference in the effect of hyperpolarization on the negative after-potential occurring during the descending portion of the spike and the end-plate potential, the two processes must be different in nature. The negative after-potential which follows the spike in the denervated part of the fiber is probably due to residual permeability changes generated by the spike potential. The descending phase of the end-plate potential, particularly in cases when it is considerably lengthened and changed in shape through calcium ion insufficiency or by phenyldiguanide, is probably due to the development of a local excitatory process in the electrically excitable membrane surrounding the end-plate. Therefore, the marked reduction of the descending prolonged end-plate potential phase induced by the influence of an anode must be thought to result from a hindrance to the spread of excitation from the membrane of the end-plate to the neighboring excitable membrane.

The next step was to determine the effect of anodic polarization on the transition of the end-plate potential into a spike. Spike potentials of a muscle fiber were taken through a double-barrelled microelectrode placed near the end-plate. When the nerve-muscle preparation was in good condition, the ascending part of the spike suffered no noticeable delay. After applying anodic polarization, besides an increase in spike potential, there was also a well shown dip corresponding to a delay in the transition of the end-plate potential into a spike. Increasing the polarizing current to $1 - 4 \cdot 10^{-7}$ a caused complete disappearance of the spike potential, although the end-plate potential was still recorded (Fig. 3, A, 3).

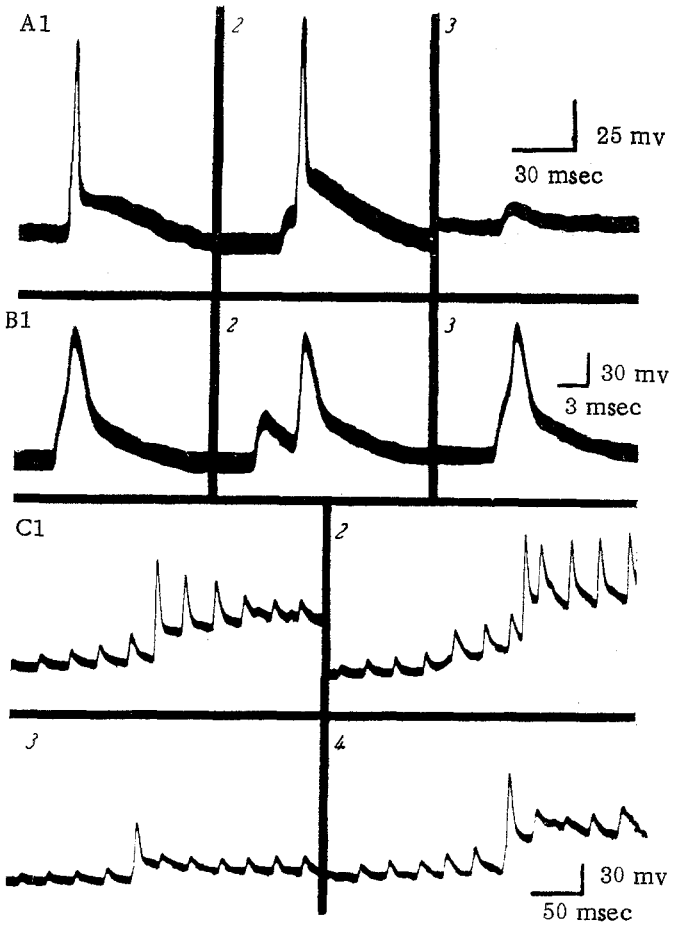

Fig. 3. The effect of polarization on single and repeated muscle fiber responses. Electrode placed near the end-plate. A: 1) normal; 2) hyperpolarizing current $8 \cdot 10^{-8}$ a; 3) hyperpolarizing current $2 \cdot 10^{-7}$ a; B: 1) normal; 2) hyperpolarizing current $6 \cdot 10^{-8}$ a; 3). normal; C: 1) normal; 2) hyperpolarizing current $8 \cdot 10^{-8}$ a; 3) depolarizing current $8 \cdot 10^{-8}$ a; 4) normal. Motor nerve stimulated at a frequency of 40 cycles.

Interference with the transmission of excitation from the end-plate on to the electrically excitable membrane was also present at strengths of hyperpolarizing current which were without effect on the amplitude of the spike potentials. A high-speed trace of this process is shown in Fig. 3, B. It can be seen that when the hyperpolarizing current is switched on, there is a scarcely noticeable dip in the ascending portion of the spike, corresponding to the transition of the local potential into a spike. With a moderate degree of hyperpolarization the time for the development of the electrical response of the electrically excitable portion of the fiber was considerably delayed. After the hyperpolarizing current had been switched off, the initial response was restored.

In connection with these results it would be interesting to determine the effect of polarization on the ability of the neuro-muscular junction to produce rhythmical stimulation. Examples of the action of hyperpolarization and depolarization on the transmission of repetitive impulses through a neuro-myal junction are shown in Fig. 3, B. It can be seen that a spike potential is not produced in response to the stimulus itself, but results from the gradual increase in the amplitude of the successive end-plate potentials (postactivational facilitation). A spike discharge occurs when the end-plate potential reaches a certain critical value. However, the amplitude of the subsequent spike discharges is rapidly reduced (pessimal effect). When there is hyperpolarization, the number of stimuli required to potentiate a spike is increased. The effect is probably due to an interference of the transmission of excitation from the end-plate on to the adjacent muscular membrane and to an increase in the critical depolarization level required for the generation of a spike. However, after this level has been reached, the spike potentials arise at each successive impulse, i. e., the pessimal effect is reduced.

On the other hand, with depolarization the critical level is reduced and the spike potential develops even more rapidly than normally, but the impulses which follow are no longer capable of generating a spike. After the polarizing current has been switched off, the initial level is restored.

Thus, with tetanic stimulation hyperpolarization may lead to a reduction, and depolarization to an increase of the pessimal effect. It must, however, be noted that the effect of polarization on the ability of the neuro-muscular junction to reproduce rhythmical impulses depends on the strength of the polarizing current. With anodic polarization of an intensity which will cause appreciable interference with the transmission of excitation from the end-plate on to the neighboring surface of the muscle fiber ($1.5 - 4 \times 10^{-7}$a), the pessimal effect was more marked than normally.

SUMMARY

Double-barrelled intracellular microelectrodes were used to determine the effect of direct-current polarization on the electrical responses of an indirectly stimulated skeletal muscle fiber membrane. Hyperpolarization increased the amplitude of spike potentials, negative after-potentials, and end-plate potentials. Depolarization had the reverse effect. The dependence of the amplitude of the electrical responses of the muscle fiber on its polarization was most pronounced when the concentration of calcium ions in the Ringer's solution was diminished. Anodal polarization interfered with the spread of excitation from the end-plate region to the adjacent fiber membrane. Polarization influences the ability of the myoneural junction to conduct the rhythmic impulses to the motor nerve.

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